RESEARCH PROPOSAL

IN VIVO APPLICATIONS OF GREEN SYNTHESIZED SILVER NANOPARTICLES AS HYDROGEL DRESSINGS

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Introduction

Nanotechnology is the modern field of science which plays a dominant role in day to day life aspects. It deals with the production, manipulation and use of material ranging in nano meters(Kavitha et.al., 2013). For the past few decades there has been a considerable research interest in the area of nanotechnology using nanoparticles such as metals, semiconductors and metal oxides are of great interest for a wide variety of applications in the field of information, energy, environmental and medical technologies. Nanotechnology offers great opportunities for improving wound treatments (Hahens *et.al.,* 2007). Although wounds are always problematic but chronic wounds as compared to acute wounds, are more troublesome. Chronic wounds are defined as wounds that fail to proceed through the normal phases of wound healing in an orderly and timely manner. In chronic wounds, bacterial colonization, biofilm production, and infection are huge global problems, compounded by the increased incidence of MDROs found in these wounds (Domenico *et.al.,* 2012). Chronic wounds have a complex microenvironment that houses multiple bacterial species. The appropriate systemic antibiotics are considered essential for the treatment of these nonhealing, clinically infected wounds, but the relevance and use of systemic and topical antibiotics in the treatment of such wounds now a matter of concern (Rahim *et.al.,* 2017). Because variety of microorganism have developed the ability to survive exposure to an antibiotic. Antibiotic and antimicrobial resistance has become a crisis in health care and, simply put, has emerged from the overuse and inappropriate use of antibiotics and antimicrobials. These resistant elements of bacteria, viruses, fungi, and parasites have made diseases that were once easily treatable deadly again. Therefore, an alternative way to overcome the antibiotic and drug resistance of various microorganisms is needed desperately, especially in medical devices, pharmaceuticals, and so forth. Silver nanoparticles exhibit remarkable biological properties, such as anti-inflammatory, antiviral activities and antibacterial properties with less bacterial resistance (Daeschlein, 2013). The biosynthesis of nanoparticles as an emerging highlight of the intersection of nano-biotechnology has received increasing attention due to a growing need to develop environmentally benign technologies in material synthesis. Generally, metal nanoparticles are synthesized through various methods including chemical and mechanical methods, electrochemical techniques, and photochemical reactions in reverse micelles. Most of these methods involve toxic chemicals (Zahir *et.al.,* 2012). Silver nanoparticles have unique

properties that find its way in different filed (Kalishwaralal *et.al*., 2010). AgNO3 were proven to be most efficient as they possess good antimicrobial and antioxidant activities (Niraimathi*,et.al.*,2012). Silver nanoparticles are widely applied to human contact areas such as medical and pharmaceutical applications. According to the above points, there is a growing need to develop eco-friendly processes for nanoparticle synthesis that do not use toxic solvents. Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. *Trachyspermum ammi* is a source of medicinally active compounds and have various pharmacological effects; (Mallikarjuna *et.al.,* 2011). Hence, it is encouraging to find its new therapeutic uses. Silver nanoparticle dressings are now the new gold standard in the conservative treatment of wounds. The drug-loaded dressings with biogenic silver nanoparticles act as a barrier to microorganisms and thus prevent secondary infections, while stimulating the wound-healing environment (Rath *et.al.,* 2016 & GhavamiNejad *et.al.,* 2016). Cancer is one of the leading causes of death in the world. Several therapies are available to treat various types of cancer. Chemotherapy in combination with cytotoxic agents is the most commonly utilized therapy to control many types of cancer (Wong *et.al.,* 2006).Nevertheless, these therapeutic approaches are linked to severe side effects, Nanoparticles play a critical role in refining the compatibility and bioavailability of natural products in the treatment of several chronic diseases, including cancer. Among the metallic nanoparticles, silver nanoparticles (AgNPs) are a popular choice in disease management because of their specific interaction with and disruption of the mitochondrial respiratory chain. AgNPs disrupt mitochondrial function by inducing the generation of reactive oxygen species and suppressing ATP synthesis, which lead to DNA damage.The present study aims to green synthesize metallic nanoparticles and their biomedical applications involving wound repair and anticancer mechanism.

Review of literature

Nanotechnology is a technique which affect specific area as well as provides the design, production and application of materials at atomic scales in order to produce new nanosized particles (Hahens *et.al.,* 2007). These are known as nanoparticles with 1 and 100 nanometres size. As nanotechnology is making an impact in every field of life, researchers are expanding their interests towards synthesis of nanoparticles as they provide superior properties for different types of biomedical applications.

Types of Nanoparticles

(a) Inorganic nanoparticles

Nanoparticles have certain physical properties that mainly include size- dependent optical, magnetic, electronic, and catalytic properties. Bio related application are involved for the preparation of these interesting nanoparticles like iron oxides, gold, silver, silica, quantum dots etc (Ladj *et.al.,* 2013). Novel physical properties mainly related because of their size approaches nanometer scale dimension (Mark Asta *et.al.,* 2007).

(b) Polymeric nanoparticles

Polymeric nanoparticle is also a type of nanoparticle. In the recent year polymeric nanoparticle has a tremendous development in the field of research. The dispersion of preformed polymers and the polymerization of monomers are two strong strategies mainly involved for preparation (Prasad Rao and Kurt Geckeler, 2011). 10 1000nm it is the range of size involved with solid structure. Nanotubes are members of the fullerene structural family. Their name is derived from their long, hollow structure with the walls formed by one-atom-thick sheets of carbon called graphene. These sheets are rolled at specific and discrete ("chiral") angles and the combination of the rolling angle and radius decides the nanotube properties.

(c) Dendrimers

Dendrimers arise from two Greek words: Dendron meaning tree and Meros meaning part. Structure of dendrimers has a well-defined size, shape and defined molecular weight and also Dendrimers are hyper-branched, globular, monodisperse, three dimensional nanoscales synthetic

Polymers. Molecular chemistry and polymer chemistry both exhibit well-defined characteristics features of Dendrites (Malik *et.al.,* 2012).

(d) Metal nanoparticles

There is a wide range of different nanoparticles which have been reported in the literature which are neither polymer nor oxide based. Many of these have been prepared by either stabilised precipitation or by micelle templating. These nanomaterials include quantum dots, nanogold, nanosilver and metal oxides, such as titanium dioxide. A quantum dot is a closely packed semiconductor crystal comprised of hundreds or thousands of atoms, and whose size is on the order of a few nanometers to a few hundred nanometers. Changing the size of quantum dots changes their optical properties.

There is a wide range of different nanoparticles which have been reported in the literature but metallic nanoparticles are one important and widely studied group of materials, showing great diversity and many different uses. In order to survive in environments containing high levels of metals, organisms have adapted by evolving mechanisms to cope with them. These mechanisms may involve altering the chemical nature of the toxic metal so that it no longer causes toxicity, resulting in the formation of nanoparticles of the metal concerned (Slavin *et.al.,* 2017). Thus nanoparticle formation is the "by-product" of a resistance mechanism against a specific metal, and this can be used as an alternative way of producing them (Raghunath *et.al.,* 2017).

Main characteristics of metal nanoparticles:

- Large surface area to volume ratio
- Large surface energies
- The transition between molecular and metallic states
- Short range ordering
- Large number of low coordination sites

Due to their unique properties that exist at nanometric scales, metal nanoparticles attain great interest and expansion.

Types of metal nanoparticles

Among these several metallic nanoparticles, Silver nanoparticles were found to be the most effective antimicrobial agent against bacteria, viruses, and other eukaryotic microorganisms (Gong, 2007). For centuries silver compounds have been exploited for their medicinal properties. Silver compounds were popular remedies for tetanus and rheumatism in the 19th century and for colds and gonorrhea before the advent of antibiotics in the early part of the 20th century (Mirsattari, *et.al.,* 2003).

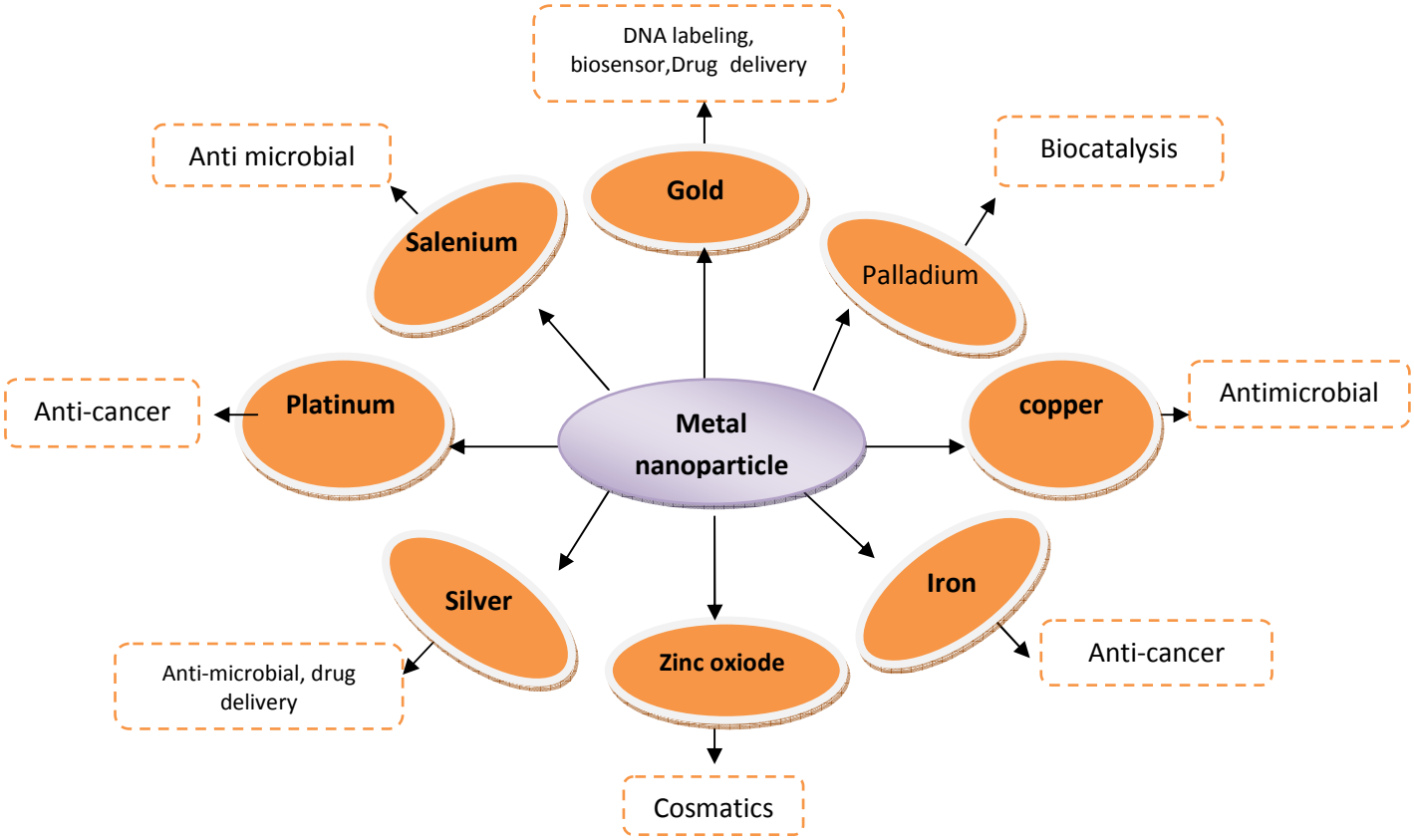


Fig 1: Types of metal nanoparticles

At present, silver has reemerged as a viable treatment option for infections encountered in burns, open wounds, and chronic ulcers. With the advances in nanotechnology, pure silver has recently been engineered into nano sized particles with diameter less than 100 nm and utilized in the treatment of wounds.

Properties of Silver Nanoparticles

(1) Silver compounds have been used in medicine throughout the history of civilization (Klasen, 2000., Lansdown, 2002., Bhattacharya, *et.al.,* 2007., & Oberdörster, *et.al.,* 2009).

(2) It is easy to synthesize silver nanoparticles in large scale by several simple, inexpensive, safe, and reliable ways, including wet chemical, physical and biological methods. (Patra, *et.al.,*2008)

(3) They can be synthesized in sizes from 2 to 500 nm by changing the reaction parameters.

(4) They can be easily synthesized in different shapes (spheres, rods, tubes, wires, ribbons, plates, cubes, hexagons, triangles) by the selection of templates and reaction conditions. (Patra, *et.al.,*2008)

(5) Because of the presence of a negative charge on their surface, they are highly reactive, which makes their surfaces modifiable by means of several biomolecules, a factor that aids in drug delivery. (Patra, *et.al.,*2008).

(6) Silver nanoparticles exhibit antibacterial effects against a large number of bacterial species.

(7) Bacterial resistance to elemental silver is extremely rare, emphasizing the presence of multiple bactericidal mechanisms acting in synergy.

(8) Silver nanoparticles can be easily incorporated in cotton fabric and dressings and have significantly decreased wound-healing time by an average of 3.35 days and increased bacterial clearance from infected wounds, with no adverse effects observed for the dressing.(Carlson, *et.al.,*2008).

(9) Anti-inflammatory properties of silver nanoparticles also promote wound healing by reducing cytokine release, (Boucher, *et.al.,* 2008) decreasing lymphocyte and mast cell infiltration.(Thirumurugan, *et.al.,* 2011).

These extraordinary properties of silver nanoparticles offer great opportunities for improving wound treatments. The nanometer scale opens the way for the development of novel materials for use in highly advanced medical technology. Silver nanoparticles exhibit remarkable

biological properties, such as anti-inflammatory, antiviral activities and antibacterial properties with less bacterial resistance which makes them more suitable for wound healing treatment.

Silver nanoparticles as an effective antibacterial agent

Silver shows some unique physical and chemical properties and owing to their high ratio of surface area to volume, silver nanoparticles have emerged as novel antimicrobial agents. Silver nanoparticles can be used in various fields, particularly medicine and pharmaceuticals, because of their low toxicity to human cells, high thermal stability, and low volatility (Shrivastava, 2007). Silver nanoparticles exhibit antibacterial effects against a large number of bacterial species. The mechanisms of action and binding of silver nanoparticles to microbes remain unclear, but it is known that silver binds to the bacterial cell wall and cell membrane and inhibits the respiration process (Lansdown, 2002) by which the chemical energy of molecules is released and partially captured in the form of adenosine triphosphate. Silver nanoparticles interact with sulfur-containing proteins of the bacterial membrane, as well as with phosphorus-containing compounds such as DNA, to inhibit replication (Shrivastava, 2007). The bactericidal effect of silver has also been attributed to inactivation of the enzyme phosphomannose isomerase (Beddy *et.al.,* 2004) which catalyzes the conversion of mannose-6-phosphate to fructose-6-phosphate, an important intermediate of glycolysis, the most common pathway in bacteria for sugar catabolism.

Mechanism of action of silver nanoparticles

The silver nanoparticles show efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Feng *et.al.,* 2000; Sondi and Salopek-Sondi, 2004; Morones *et.al.,* 2005; Song *et.al.,* 2006).

Biogenic Silver nanoparticles: Green synthesis

Silver nanoparticles are widely applied to human contact areas such as medical and pharmaceutical applications. According to the above points, there is a growing need to develop eco-friendly processes for nanoparticle synthesis that do not use toxic solvents. Biological routes of nanoparticles synthesis using microorganism, enzymes, and plants or plant extracts have been suggested as possible eco-friendly alternatives to other methods (Zahir *et.al.,* 2012). The usage of plants for NP synthesis because it omit the process of maintaining cell cultures is more simple, faster, reliable and cost effective over other biological processes and also can be suitably for up scaling synthesis of nanoparticles (Nabikhan *et.al.,* 2010).

Hydrogels

The uncontrolled use of conventional antibiotics has led various pathogens to become drug resistant and to solve this problem there is an urgent need to develop new antimicrobial materials and their relevant formations. There are several classes of materials which have emerged as substitute of conventional antibiotics for example cationic polymers (Engler *et.al.,* 2012), antimicrobial peptide (Fox *et.al.,* 2013), antimicrobial nanoparticles (Huh *et.al.,* 2012) and polymeric hydrogels. Nowadays hydrogels are of great interest because they are widely used in many biomedical applications like tissue engineering (Naahidi, *et.al,*2017), drug delivery (Wei, *et.al,*2017), cell culture (Luo, *et.al,*2017), wound healings (Annabi*et.al,*2017), and so on. Hydrogels are natural or synthetic polymers which form 3D network and are capable of retaining a large amount of water and biological fluid in their swollen form (Varaprasad *et.al.,* 2017). Hydrogels are widely researched as an antimicrobial agent because of their great biomedical significance. There is another reason for using hydrogels instead of antibiotics, that conventional antibiotics act on specific molecular target such as the inhibition of protein synthesis of microbes whereas hydrogels act upon the entire cellular domain of microbial membrane to generate pores , that ultimately leads to the cell rupture, leaking of cytoplasm and finally death of the cell (Brogden, 2005 & Ng *et.al.,* 2005). Although nanoparticles posses unique characteristics which make them available for many biomedical applications but the problem arises regarding their safety when they come in contact with biological system. One possible solution to the problem is

to incorporate nanoparticles into a hydrogel matrix, resulting in decreased risk to human health and the environment.

Biomedical applications of biosynthesized silver nanoparticles

In recent years research involving NPs has generated a great deal of interest from scientists and engineers of nearly all disciplines. Among different nonmaterial employed for biomedical research, metallic NPs have proved to be the most convenient and suitable. Based on their unique optical, electrical, magnetic properties, specific heats, melting points, and surface reactivates, metallic NPs have found significant applications in a wide spectrum of biomedical utilities like imaging, sensing, drug delivery and gene targeting, wound healing etc (Khan *et.al.,* 2006).

Wound:

A wound is a breakdown in the protective function of skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue (Leaper *et.al.,* 1998). Wounds can be accidental, pathological or post-operative. There are two types of wounds: acute and chronic. Acute wounds, heal in normal, orderly sequence of healing, are either traumatic or surgical and move through the healing process at a predictable period from injury to complete wound closure. Chronic wounds fail to progress through a normal, orderly and timely sequence of repair and that has not resolved over a reasonable period of time and they do not progress through the predictable stages of wound healing. These wounds may eventually pass through the repair process without restoring sustained anatomical and functional results (Nancy Broderick, 2009).

Normal/ acute wound healing cascade

Wound healing is a complicated procedure involving a combination of activities of different tissues and cell lineages, divided into three differential phases: the coagulation and inflammatory phase, the proliferation and tissue formation phase, and the maturation and remodeling phase. The phases are not separated but rather they overlap and influence each other. The healing phases are strictly followed by acute wounds, which progress through each stage towards successful epithelialization and wound closure (Rahim *et.al.,* 2017). The coagulation/inflammatory phase start straight after the injury in an attempt to minimize the damage that occurred. It involves platelet adherence to the damaged vessels and aggregation,

blood clotting, and immune activation. Thrombocytes will immediately start to produce different cytokines, chemokines, and growth factors (Demidova *et.al.,* 2012). Among the released growth factors, platelet-derived growth factor (PDGF) and transforming growth factors alpha 1 and alpha 2 (TGF-α1, TGF-α2) are involved in attracting inflammatory cells (Demidova *et.al.,*2012). In the early steps, local dendritic cells express pattern recognition receptors, such as Toll-like receptors (TLRs) that recognize the pathogen-associated molecular patterns (PAMPs) of colonizing microbes. This interaction leads towards innate immune activation, characterized by a significant recruitment of neutrophils and monocytes to the site of damage (Tsiouris *et.al.,* 2017). The reactive oxygen species released by the attracted leukocytes are essential for the antimicrobial action at the wound site (Demidova *et.al.,*2012). The complement system is also activated (Jahromi *et.al.,* 2018). The initial changes are followed by the transformation of monocytes into macrophages and the migration of fibroblasts, leukocytes, keratinocytes, and endothelial cells, as well as by the local accumulation of growth factors, to the wound (Jahromi *et.al.,* 2018). Later on, there is a change towards adaptive immunity involving lymphocytes (T-cells and B-cells) and a complex cascade of cytokines and chemokines (Jahromi *et.al.,* 2018). This leads to the second phase of proliferation and tissue formation, which includes three important steps: re-epithelialization, angiogenesis, and development of granulation tissue. Local cytokines and growth factors induce re-epithelialization by the activation of fibroblasts, keratinocytes, epithelial cells, and stem cells. Moreover, fibroblasts produce collagen and other molecules that form a new extracellular matrix, an appropriate scaffold for cell adhesion, growth, and differentiation (Demidova *et.al.,* 2012). The extracellular matrix is composed of fibronectin, fibrin, hyaluronic acid, and collagen type III at the beginning replaced later with collagen type I (Demidova *et.al.,*2012). To support all the cells involved in the healing process, the blood supply plays a key role; this is why angiogenesis starts immediately after a blood vessel injury occurs (Demidova *et.al.,*2012). During angiogenesis, multiple endothelial growth factors such as PDGF, vascular endothelial growth factor (VEGF), fibroblast growth factor beta (FGF-β), and granulocyte-macrophage colony-stimulating factor (GM-CSF) act on the development of new blood vessels, with an increase in nutrient and oxygen availability to the developing tissue (Jahromi *et.al.,* 2018). This is a very complex process involving, among others, the degradation of the basement membrane of the blood vessels, the migration of the endothelial cells to the wound site, and the formation of new blood vessels, all this under the influence of different

substances, factors, and even cell progenitors, such as the endothelial progenitor cells (Leone *et.al.,* 2011). At the end of this phase, the fibroblasts differentiate into myofibroblasts or undergo apoptosis (Demidova *et.al.,*2012). Finally, tissue maturation and remodeling occur with a change in composition of primarily type I collagen that produces the mature scar (Rahim *et.al.,* 2017). In this phase, cellular apoptosis plays an essential role (Rai *et.al.,* 2005). If the apoptotic process is malfunctioning, several wound healing pathologies can develop, such as hypertrophic scars or keloids (Demidova *et.al.,* 2012).

It is important to mention that the cutaneous injury can lead to neuroendocrine changes with the secretion of stress mediators, such as cortisol, epinephrine, norepinephrine, acetylcholine, catestatin, substance P, and α-melanotropin (Tsiouris *et.al.,* 2017). It was stated that these molecules alter the cutaneous microbiota with a prevalence of harming bacteria contributing to wound infection and impaired healing (Tsiouris *et.al.,* 2017).

Pathophysiology of chronic wound repair

Chronic wounds are defined as wounds that fail to proceed through the normal phases of wound healing in an orderly and timely manner. Often, chronic wounds stall in the inflammation phase of healing. Despite differences in etiology at the molecular level, chronic wounds share certain common features, including excessive levels of proinflammatory cytokines, proteases, ROS, and senescent cells, as well as the existence of persistent infection, and a deficiency of stem cells that are often also dysfunctional. Failure to rebuild the skin integrity may be due to a large number of potential contributory causes. Local factors that affect tissue oxygenation or inflammation—vascular disease (arterial or venous chronic pathologies, phlebitis, thrombosis, vasculitis), immobility, severe trauma or repeated injuries in the same region, chronic pressure, chronic edema, colonization by opportunistic pathogens, chronic infection with the development of biofilms, and others can increase the risk of healing failure (Tsiouris *et.al.,* 2017). However, systemic factors that affect the overall health of the individual can play an important role in the healing process (for example, diabetes, diet and obesity, hypertension, chronic inflammatory diseases, and others) (Tsiouris *et.al.,* 2017).

Chronic wounds fail to heal in the physiologically ordered sequence of events, most of them entering a vicious cycle of inflammation and infection (Sebastian *et.al.,*2015). Non-healing

wounds display important morphological, clinical, biochemical, and microbiological differences when compared with both acute and healing wounds (Kirker *et.al.,* 2017). The tissue damage and repair are misbalanced, resulting in the ulcer's lodging in a particular wound stage, most frequently in the inflammation or granulation stages, and consequently impairing progression toward wound healing (Demidova *et.al.,*2012). Studies have shown that cells from chronic wounds have lower multiplying rates and are similar to senescent ones (Sebastian *et.al.,*2015). Moreover, the fibroblasts from chronic wounds are less responsive to PDGF and transforming growth factor beta (TGF-β) (Sebastian *et.al.,*2015). Another characteristic of chronic wounds is the reduced angiogenesis and, hence, a low blood supply that results in hypoxia (Sebastian *et.al.,*2015).Other factors involved in the elongation of the healing process are an increased concentration of iron that results from the destroyed erythrocytes and mitochondrial dysfunction (Sebastian *et.al.,*2015).It was stated that a hallmark of chronic wounds is represented by the insufficient bioavailability of growth factors, critically important for normal injury repair; the deficiency is probably caused by diminished synthesis and/or excessive degradation (Demidova *et.al.,*2012). This observation might suggest the potential use of exogenous growth factors in the treatment of non-healing wounds (Demidova *et.al.,*2012).

Microbial colonization and wound healing

One significant factor that influences the healing process of wounds is characterized by the microbial colonization of the wound. Although initially the host's immune system controls the proliferation of bacteria, further on the microorganisms develop biofilms and a tolerance toward immune defense mechanisms. Therefore, a microbial infection may occur, which could lead to impaired wound healing (Percival, 2017).

The exact role of bacteria in delayed wound healing remains controversial because there is a discrepancy in the published scientific results. It was previously believed that high microbial levels are correlated with the inability to heal. It was stated that wound infection risk is proportional to the number of colonizing microbes (N) multiplied by their virulence (V) and divided by host immunity (I):

$$\text{Infection risk} = (N \times V)/I$$

Several factors could influence each of these variables: environmental factors such as temperature and humidity; wound characteristics such as depth, vascularization; patient compliance to treatment by applying proper care and cleaning of the wound. On the other hand, recent research has shown that an association between bacteria and delayed healing does not always exist, and that bacteria might be responsible in some situations of a positive wound outcome, for example, after the topical contact with bacterial lipopolysaccharides (Mihai, 2015 & Wong *et.al.,*2013). It was suggested that the qualitative characteristics of the microbial ecosystem can modulate its impact on wound healing (Wong *et.al.,*2013).

Microbial biofilms

Despite the focus of microbiological research on planktonic (single organisms, suspended/free floating) populations of bacteria, it has become apparent that bacteria exist predominately within biofilms in natural and clinical settings (Costerton *et.al.,* 1987). Research over the past few decades has now clearly established that 99.9% of all known microorganisms in natural settings are attached to surfaces, due to the nutritional and protective benefits associated with life in an adherent population (Cowan, 2011). Biofilms are three-dimensional mosaic consortia of microbes, which accumulate and organize at surfaces within an extracellular polymer (Wilking *et.al.,* 2013). Once bacteria attach to a surface, they produce complex exopolymers—containing polysaccharides, proteins, and nucleic acids (Branda *et.al,*2005).which help both to preserve their attachment to the surface and to maintain bacterial cells in close proximity to each other. As the number of bacteria present in a specific area increases, this adherent population is referred to as a biofilm. The vast majority of the biofilm's volume, 80%–85%, is comprised of the exopolymer, with the remaining 15%–20% consisting of microorganisms (Cowan, 2011). The high content of water in a biofilm contributed to the challenge of its original observation, as the otherwise unsupported exopolymer collapses onto the bacterial surface during dehydration for electron microscopy (Cowan, 2011).

Mechanisms of antibiotic resistance and implications for health care

Antibiotics target one or multiple modes of cellular communication which allow microorganisms to proliferate. These include cell wall, membrane transport, RNA function, DNA synthesis, protein function, or enzyme activity. Interrupting cellular communication and thus proliferation

has made antibiotics very effective against a broad range of microoganisms. In looking at the history of multidrug-resistant organisms (MDROs) we must remember that there are two sides to every coin, and with the positive side of clinical efficacy against microoganisms there is also a downside. To ensure their survival, it has become necessary for microorganisms to evolve and genetically mutate. These processes have caused the organisms of today to be much different from the organisms of yesterday, much more virulent, and more multidrug resistant.

Antibiotic and antimicrobial resistance has become a crisis in health care and, simply put, has emerged from the overuse and inappropriate use of antibiotics and antimicrobials. These resistant elements of bacteria, viruses, fungi, and parasites have made diseases that were once easily treatable deadly again. We must understand the difference as well because antimicrobial resistance refers to the loss of effectiveness across the entire range of organisms, whereas antibiotic resistance refers only to bacterial resistance to medications. As rapidly as new generations of antibiotics and antimicrobials are developed, the genetics of microorganisms change to enhance their resistance. With this come higher health care costs, poorer outcomes, and a higher risk of death for patients infected with resistant strains.

Specific Multidrug-Resistant Pathogens

Multidrug-resistant organisms (MDROs) are increasingly implicated in both acute and chronic wound infections. The limited therapeutic options are further compromised by the fact that wound bacteria often co-exist within a biofilm community which enhances bacterial tolerance to antibiotics. As a consequence, topical antiseptics may be an important consideration for minimising the opportunity for wound infections involving MDROs. MDROs are bacteria, fungus, yeast, and viruses that have developed resistance to antimicrobial drugs. The most common include the following:

- MRSA – Methicillin/oxacillin-resistant *Staphylococcus aureus*
- VRE – Vancomycin-resistant *enterococcus*
- ESBLs – Extended-spectrum beta-lactamases

- PRSP – Penicillin-resistant *Streptococcus pneumoniae*
- MDRTB – Multidrug-resistant tuberculosis

Weakness of current wound therapy

There are a number of available therapeutic options, which aim to prevent and treat infections as well as they promote healing and prevent scarring. Most infections can be prevented with the correct prophylaxis; however, high antibiotic pressure has selected for highly resistant bacteria. Although it represents the first line of treatment in acute infections, antibiotic therapy administered systemically or topically in non-toxic doses cannot eradicate biofilm infections and there is no evidence to support its further use. Biofilm antibiotic susceptibility testing revealed that due to microbial tolerance, bactericidal concentrations reached values 1000 times higher compared to the ones for planktonic bacteria (Domenico *et.al.,* 2012). There is a fine line between the beneficial effects of antibiotic therapy, such as the eradication of planktonic bacteria and biofilm disruption, and its pathogenic effects, such as the development of bacterial resistance and tolerance to antibiotics. There are several experimental studies that brought proof on the fact that fluoroquinolones, tetracycline, rifampin, daptomycin, and vancomycin can rapidly diffuse into the deeper levels of the biofilms and contribute to their disruption (Domenico *et.al.,* 2017), but these results are achieved only with other therapeutic approaches (topical antiseptics, wound debridement, and others). Recent guidelines on wound care emphasize the importance of topical antiseptic therapy and personalized dressings. Although antiseptics such as povidone-iodine, chlorhexidine, hydrogen peroxide, boric acid, silver sulfadiazine or nitrate, sodium hypochlorite, and mafenide acetate are intrinsically cytotoxic to bacteria, fungi, and other microorganisms, they might also damage host cells and adversely affect wound healing (Jahromi *et.al.,* 2018). Octenidine dihydrochloride and polyhexamethylene biguanide (polyhexanide) seem to be the most efficient and well-tolerated antiseptics (Daeschlein, 2013), while the latter is considered the best promoter of wound healing (Hoiby *et.al.,*2005).

Another alternative which is available for wound healing is polymicrobial vaccine. Polymicrobial vaccines have not proven to be as efficient as expected in clinical trials. Bertesteanu et al. (2014) proposed an optimization of the vaccines: to target shared microbial

determinants and to simultaneously attenuate the potential virulence of different co-infecting species in order to reduce the risk of recurrence (Bertesteanu *et.al.,* 2014).

World health organization priority pathogens report

In response to the global crisis of MDROs and to promote the research and development of new antibiotics, the WHO published the 2017 Priority Pathogens report. In the report pathogens are divided into three categories: critical, high, and medium priority. The goal of the report was to focus on the need for development of new antibiotics to treat drug-resistant bacterial infections and tuberculosis. It also noted that once completed, the same process should be followed for viral, fungal, and parasitic infections.

The report also stated that this must be a high-priority item in the global political agenda of world leaders and health policy makers linked to the development of appropriate health care delivery services and stewardship that will safeguard the use of current and future medications. The committee stressed that the only possible defense against the global crisis of antimicrobial and antibiotic resistance is a targeted and coordinated global effort by all stakeholders on a mission to ensure health for all.

Silver nanoparticles as wound healing agent

The ultimate goal for wound healing is a speedy recovery with minimal scarring. Wound healing proceeds through an overlapping pattern of events including coagulation, inflammation, proliferation, matrix, and tissue remodeling. The availability of AgNPs has ensured a rapid adoption in medical practice. Their application can be broadly divided into diagnostic and therapeutic uses. In terms of therapeutics, one of the most well-documented and commonly used applications of AgNPs is in wound healing. Compared with other silver compounds, many studies have demonstrated the superior efficacy of AgNPs in time required for healing. Silver nanoparticles exert positive effects on wound through their antimicrobial properties, reduction in wound inflammation, and modulation of fibrogenic cytokines. Wound-healing property of silver nanoparticles is due solely to their antimicrobial property, confirmed that silver nanoparticles are a more effective antibacterial agent. Wounds that are treated with silver nanoparticles show fast recovery. Wounds treated with AgNPs, show better collagen alignment after healing when

compared to controls, which resulted in better mechanical strength, although the exact mechanisms for these biological effects have not yet been elucidated (Kwan, *et.al.,* 2011) .

Wound dressing impregnated with silver nanoparticles

Impaired wound healing deals with the major problem of infection. Rather than this there should be a medium which maintain a moist environment around the wound, permit diffusion of gases, protect wound from microbes and not contaminate the wound with foreign particles, Provide mechanical protection and control local temperature and pH of wound area. These complications spurred the search for drug-loaded dressings, which can overcome such problems (Anjaiah *et.al.,* 2001). Drug-loaded dressings are prepared by incorporating drugs in the dressings. When applied to a wound, drug-loaded dressings act as a barrier to microorganisms and thus prevent secondary infections, while stimulating the wound-healing environment. Among the various antimicrobial agents, silver nanoparticles have shown strong inhibitory and antimicrobial activity and have no negative effect on the human body (Panyala *et.al.,* 2008). These particles can be incorporated into several kinds of materials, such as clothes. Clothes incorporating with silver nanoparticles are sterile and can be used to prevent or to minimize infection with pathogenic bacteria. Nowadays, metal-based topical dressings have been widely used as a treatment for infections in burns, open wounds, and chronic ulcers (Lansdown, 2002).

Skin Cancer:

Skin cancer is the uncontrolled growth of abnormal skin cells. It occurs when unrepaired DNA damage to skin cells (most often caused by ultraviolet radiation from sunshine or tanning beds) triggers mutations, or genetic defects, that lead the skin cells to multiply rapidly and form malignant tumors.

There are three major types of skin cancer:

(1) **Basal cell carcinoma** (the most common)- This is the most common cancer in humans. Over 1 million new cases of basal cell carcinoma are diagnosed in the U.S. each year. There are several different types of basal cell carcinoma, including the superficial type, the least worrisome variety; the nodular type, the most common; and the morphed form, the most challenging to treat

because the tumors often grow into the surrounding tissue (infiltrate) without a well-defined border.

(2) **Squamous cell carcinoma** (the second most common), which originate from skin cells, accounts for about 20% of all skin cancers but is more common in immunosuppressed people. In most instances, its biologic behavior is much like basal cell carcinoma with a small but significant chance of distant spread.

(3) **Melanoma,** which originates from the pigment-producing skin cells (melanocytes) but is less common, though more dangerous, than the first two varieties.

Other rare forms of skin cancer include lymphomas, Merkel cell cancers, and cancers of other tissue in the skin, including sarcomas as well as hair and sweat gland tumors (Gary *et.al.,* 2014).

Signs and symptoms of skin cancer

Most basal cell carcinomas have few if any symptoms. Squamous cell carcinomas may be painful. Both forms of skin cancer may appear as a sore that bleeds, oozes, crusts, or otherwise will not heal. They begin as a slowly growing bump on the skin that may bleed after minor trauma. Both kinds of skin cancers may have raised edges and a central ulceration.

Signs and symptoms of basal cell carcinomas include:

- Appearance of a shiny pink, red, pearly, or translucent bump
- Pink skin growths or lesions with raised borders that are crusted in the center
- Raised reddish patch of skin that may crust or itch, but is usually not painful

Signs and symptoms of squamous cell carcinomas include:

- Persistent, scaly red patches with irregular borders that may bleed easily
- Open sore that does not go away for weeks
- A raised growth with a rough surface that is indented in the middle
- A wart-like growth

The most common sites where skin cancer develops

Skin cancers typically arise in areas of the skin exposed to the sun repeatedly over many years such as on the face and nose, ears, back of the neck, and the bald area of the scalp. Less commonly, these tumors may appear at sites with only limited sun exposure such as the back, chest, or the extremities. However, skin cancer may occur anywhere on the skin (Gary *et.al.,* 2014).

Complications non-melanoma skin

If you've had non-melanoma skin cancer in the past, there's a chance the condition may return. The chance of non-melanoma skin cancer returning is increased if the previous cancer was large in size and high grade (severe).If the cancer team feels there's a significant risk of non-melanoma skin cancer returning, you'll probably need regular check-ups to monitor your health. It's also important to be aware that if you've had a non-melanoma skin cancer, your risk of developing another one in the future is increased because these cancers are often multiple. This means it's important to regularly examine your skin to check for new tumors.

Green synthesized silver nanoparticles as anticancer agent

Cancer is one of the leading causes of death in the world. According to the 2014 World Cancer Report, approximately 14 million new cancer cases and 8.2 million cancer-related deaths were reported in 2012. Among the different types of cancer, lung cancer is associated with the greatest mortality (1.5 million deaths), followed by liver (745 000 deaths), stomach (723 000 deaths), colorectal (694 000 deaths), skin cancer (521 000 deaths), and esophageal cancer (400 000 deaths) (World cancer report 2014,).

Several therapies are available to treat various types of cancer. Chemotherapy in combination with cytotoxic agents is the most commonly utilized therapy to control many types of cancer (Wong *et.al.,* 2006).Nevertheless, these therapeutic approaches are linked to severe side effects, especially multidrug resistance (MDR) (Gottesman *et.al.,* 2002). There are various undesirable side effects of chemotherapy alone or in combination with cytotoxic drug therapy or radiation therapy (Komarov *et.al.,* 1999). Based on these undesirable side effects, the National Cancer

Institute (USA) has encouraged the investigation of the potential antitumor activities of plant extracts (Cai *et.al.,* 2004).

Natural compounds isolated from medicinal plants are believed to be promising leads in the development of anticancer drugs. Screening of medicinal plants and their active constituents for various biological activities, such as anticancer activity, has been a major interest since the 1960s (Cai *et.al.,* 2004).. Medicinal plants have shown activity against various metabolic diseases and cancers. However, because of their minimal size, green-synthesized nanoparticles from medicinal plants have become a keen interest of researchers.

Use of *Trachyspermum ammi* plant for green synthesis of silver nanoparticles

Trachyspermum ammi commonly known as 'Ajwain' is distributed throughout India and is mostly cultivated in Gujarat and Rajasthan. The fruit possesses stimulant, antispasmodic and carminative properties and is used traditionally as an important remedial agent for flatulence, atonic dyspepsia, diarrhea, abdominal tumors, abdominal pains, piles, and bronchial problems, lack of appetite, galactogogue, asthma and amenorrhoea. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive, cytotoxic, hypolipidemic, antihypertensive, antispasmodic, broncho-dilating actions, antilithiasis, diuretic, abortifacient, antitussive, nematicidal, anthelmintic and antifilarial(Bairwa *et.al.,* 2012). Further, studies reveal the presence of various phytochemical constituents mainly carbohydrates, glycosides, saponins, phenolic compounds, volatile oil (thymol, γ-terpinene, para-cymene, and α- and β-pinene), protein, fat, fiber and mineral matter containing calcium, phosphorous, iron and nicotinic acid (Bairwa *et.al.,* 2012). These studies reveal that *T. ammi* is a source of medicinally active compounds and have various pharmacological effects; hence, it is encouraging to find its new therapeutic uses.Plant extract coated nanoparticles, has many medical advantages it can be used in drugs and cosmetic applications. It consists of the fatty acids, proteins, flavonoids and alkaloids that promotes antioxidant, antimicrobial, anti inflammatory and immune stimulant activity(Lather *et.al.,* 2010). This method of synthesis of metal nanoparticles using TA extract is a low cost approach at ambient condition.

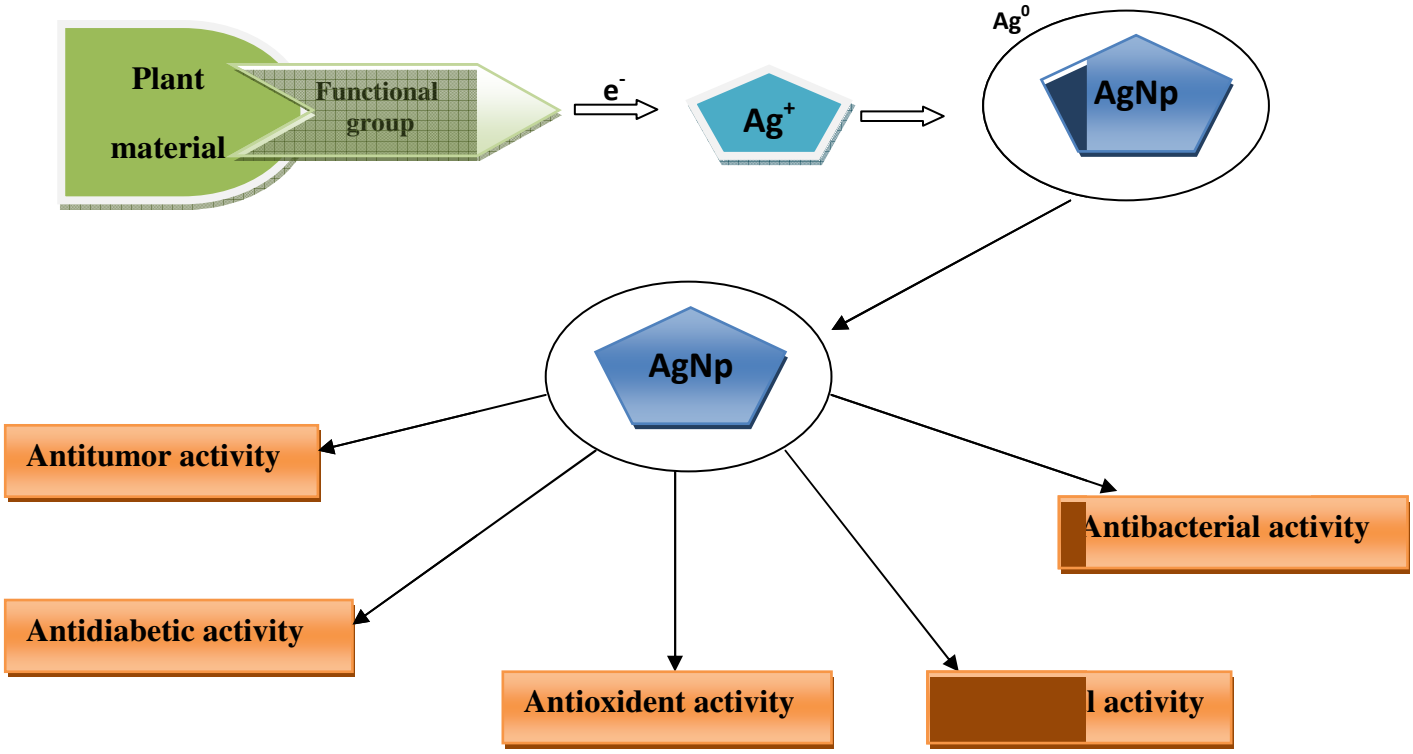
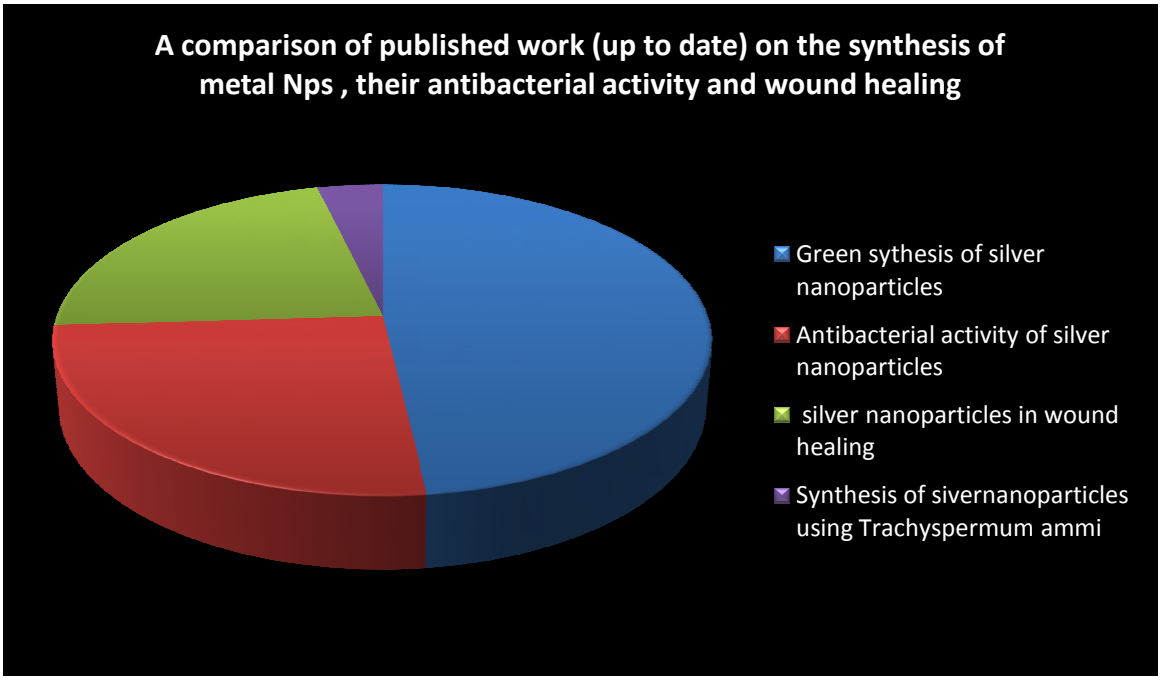


Fig 3: Biogenic synthesis of nanoparticles and their biological activities (Rao *et.al.*, 2016)


A comparison of published work (up to date) on the synthesis of metal Nps , their antibacterial activity and wound healing
Green sythesis of silver nanoparticles
Antibacterial activity of silver nanoparticles
silver nanoparticles in wound healing
Synthesis of sivernanoparticles using Trachyspermum ammi

Green synthesis of silver nanoparticles

Sr.No.	Authors	Year	Aspect(s) Studied
1.	Arya *et.al.*	2017	Green synthesis of silver nanoparticles using Prosopis juliflora bark extract: reaction optimization, antimicrobial and catalytic activities.
1.	Baghizadeh *et.al.*	2017	Green synthesis of silver nanoparticles using seed extract of Calendula officinalis in liquid phase Green synthesis of silver nanoparticles using seed extract of Calendula officinalis in liquid phase.
2.	Guilger *et.al.*	2017	Biogenic silver nanoparticles based on trichoderma harzianum: Synthesis, characterization, toxicity evaluation and biological activity
3.	Bhati *et.al.*	2014	Biosynthesis of silver nanoparticles using fresh extracts of Tridax procumbens Linn
4.	Mittal *et. al.*	2013	Synthesis of metallic nanoparticles using plant extracts
5.	Patil *et al.*	2012	Larvicidal activity of silver nanoparticles synthesized using Pergularia daemia plant latex against Aedes aegypti and Anopheles stephensi and nontarget fish Poecillia reticulate
6.	Saxena *et. al.*	2012	Green synthesis of silver nanoparticles using aqueous solution of Ficus benghalensis leaf extract and characterization of their antibacterial activity

7.	Bunghez *et. al.*	2011	Green synthesis of silver nanoparticles obtained from plant extracts and their antimicrobial activities
8..	Huang *et. al.*	2011	Biogenic silver nanoparticles by Cacumen platycladi extract: Synthesis, formation mechanism and antibacterial activity
9.	Singhal *et al.*	2011	Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity
10.	Srivastava *et al.*	2011	Plant mediated synthesis of silver nanoparticles using a bryophyte: Fissidens minutus and its anti-microbial activity
11.	SudhaLakshmi	2011	Green synthesis of silver nanoparticles from Cleome viscosa: Synthesis and antimicrobial activity
12.	Singh *et.al.*	2010	Green synthesis of silver nanoparticles using Argemone mexicana leaf extract and evaluation of their antibacterial activity
13.	Jain *et.al.*	2009	Synthesis of plant - mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities

Antibacterial activity of silver nanoparticles

Sr.No.	Authors	Year	Aspect(s) Studied
1.	Goyal *et.al.*	2018	Antibacterial, anticancer and antioxidant potential of silver nanoparticles engineered using *Trigonella foenum* seed extract

2.	Arya *et.al.*	2018	Biosynthesis and in vitro Antimicrobial Potential of Silver Nanoparticles Prepared using *Dicoma tomentosa* Plant Extract.
3.	Salomoni *et.al.*	2017	Antibacterial effect of silver nanoparticles in *Pseudomonas aeruginosa*.
4.	Chen *et.al.*	2016	Enhancement of the Antibacterial Activity of Silver Nanoparticles against Phytopathogenic BacteriumRalstonia solanacearum by Stabilization.
5.	Losasso *et.al.*	2014	Antibacterial activity of silver nanoparticles: sensitivity of different *Salmonella*serovars.
6.	Zhou *et.al.*	2012	Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and *bacillus Calmette-Guérin.*
7.	Prasad *et al.*	2011	Biogenic synthesis of silver nanoparticles using *Nicotiana tobaccum* leaf extract and study of their antibacterial effect.
8.	Savithramma *et.al.*	2011	Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants.

Silver nanoparticles in wound healing

Sr.No.	Authors	Year	Aspect(s) Studied
1.	Araújo *et.al.*	2017	The use of nanoparticles in wound treatment : a systematic review
2.	Adibhesami *et.al.*	2017	Effects of silver nanoparticles on *Staphylococcus aureus* contaminated open wounds healing in mice: An experimental study

2..	Akila *et.al.*	2014	In-Vivo Wound Healing Activity of Silver Nanoparticles : An Investigation
3.	Thirumurugan *et.al.*	2011	Superior wound healing effect of topically delivered silver nanoparticles formulation using ecofriendly potato plant pathogenic fungus: Synthesis and characterization
4.	Liu *et.al.*	2010	Silver nanoparticles mediate differential responses in keratinocytes and fibroblasts during skin wound healing
5.	Tian *et.al.*	2007	Topical delivery of silver nanoparticles promotes wound healing
6.	Atiyeh *et.al.*	2007	Effect of silver on burn wound infection control and healing : Review of the literature

Synthesis of silver nanoparticles using *Trachyspermum ammi* leaves

Sr.No.	Authors	Year	Aspect(s) Studied
1.	Sahu *et.al.*	2015	Antimicrobial activity of Trachyspermum ammi leaves mediated silver nanoparticles : Green approach

Research Gap

Review of literature suggested that metal nanoparticles are synthesized through various methods including chemical and mechanical methods, electrochemical techniques, and photochemical reactions in reverse micelles. Most of these methods involve toxic chemicals. So there is a growing need to develop biologically inspired nanoparticles synthesis process that does not use toxic chemicals in the conversion method. These biosynthesized nanoparticles may also be effective in healing of chronic wounds. The active compounds isolated from medicinal plants may not specifically function as anticancer agents or drugs but may provide alternatives for the advancement of prospective cytotoxic agents. As research progresses, new technologies will aid in the improvement of the anticancer activities of drugs.

Objective of the study

The objective of this study is –

- To characterize biogenic metal nanoparticles from herbal extract.
- To analyze the antimicrobial activity of green synthesized metal nanoparticles against gram positive and gram negative bacteria.
- To examine the effect of metal nanoparticles in topical wound healing.
- To examine the anticancer activity of green synthesized metal nanoparticles.

Methodology

Preparation of the Plant extract

Seeds of *Trachyspermum ammi* plant will be used to make the aqueous extract. Seeds will be thoroughly washed in distilled water and will be boiled into sterile distilled water and filtered through Whatman No.1 filter paper.

Preparation of metal nanoparticles

Trachyspermum ammi mediated AgNPs will be prepared in an Erlenmeyer flask by the addition of silver nitrate ($AgNO_3$) at different concentrations dropwise to the extract in different ratios with magnetic stirring at different incubation temperatures. The stirring will be continued till the change in color indicating the reduction of silver ions. The nanoparticles thus formed will be subjected to centrifugation at 10,000 rpm, for 10 min followed by removal of supernatant containing excessive silver ions (refrigerated centrifuge). Finally, the pellet will be resuspended in double distilled water and subjected to UV-vis spectrophotometric analysis (Bhati *et.al.,* 2013).

Objective 1: To characterize biogenic metal nanoparticles from herbal extract.

UV-Vis Spectra analysis

The reduction of pure M+ ions will be monitored by measuring the UV-Vis spectrum of the reaction medium after few hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis will be done by using UV-Vis spectrophotometer.

XRD measurement

The metal nanoparticles solution thus obtained will be purified by repeated centrifugation for few minutes followed by redispersion of the pellet of metal nanoparticles into deionized water. After freeze drying of the purified metal particles, the structure and composition will be analyzed by XRD and SEM.

SEM analysis

Scanning Electron Microscopic (SEM) analysis will be done. Thin films of the sample will be prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution will be removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp.

FTIR analysis

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution after reaction will be centrifuged and the resulting suspension will be redispersed in sterile distilled water. The centrifuging and redispersing process will be repeated. Thereafter, the purified suspension will be freeze dried to obtain dried powder. Finally, the dried nanoparticles will be analyzed by FTIR.

Objective 2: To analyze the antimicrobial activity of green synthesized metal nanoparticles

Antibacterial assays

The antibacterial assays will be done for Gram negative as well as Gram positive strains by standard disc diffusion method. Luria Bertani (LB) agar medium will be used to cultivate bacteria. Fresh overnight cultures of inoculums of each culture will be spread on to LB agar plates. Sterile paper discs (containing silver nanoparticles) along with four standard antibiotic containing discs will be placed in each plate.

Excision wound model

The animal (Swiss albino mice) will be anesthetized locally with injection of ketamine (80 mg/kg body weight)/xylazine (16 mg/kg body weight). The dorsal fur of the animal will be shaved. The shaved area will be swabbed with alcohol and an area of 1x1cm2 (approximate) will be demarcated on the midline of the shaved area. The marked skin will be excised with the help of a scalpel and scissors to the depth of loose subcutaneous tissue (Dhall *et.al.,* 2014).

Chronic Wound Model

Swiss albino mice will be anesthetized locally with injection of ketamine (80 mg/kg body weight)/xylazine (16 mg/kg body weight). The dorsal fur of the animal will be shaved. The shaved area will be swabbed with alcohol. To generate chronic wound full thickness 7mm diameter excision wound on dorsum of 6-7 month old mice will be performed. Twenty minutes prior to wounding, mice will be treated once intraperitoneally (IP) with 3-amino-1,2,4-triazole (ATZ) an inhibitor for catalase. Immediately after wounding, the wounds will be covered with tegaderm to prevent contamination and will be kept covered for the duration of the experiments (Dhall *et.al.,* 2014).

Objective 3: To examine the effect of metal nanoparticles in topical wound healing

Bioactivity of green synthesized metal nanoparticles

To examine the effect of metal nanoparticle in wound healing Swiss albino mice of either sex weighing 35-40 gm will be used. Animals will be divided into 6 groups. Group I will be served as control, Group II will be treated as positive control for excision wound model and group III, IV, V, VI will be served as test groups which will be received NPs. Similarly groups designation will be done for anticancerous study involving mice model for two stage skin papillomagenesis.

- **Morphometric assessment of wound re-epithelization and wound closure**

  Morphometric assessment of wound re-epithelization and wound closure will be examined and documented daily using digital photography. Wound areas (cm2) will be calculated using following formula:

$$\% \text{ Wound area} = \frac{\text{Original wound area at day 0}}{\text{Wound area on day n}} \times 100\%$$

- **Tissue Collection**

  Tissue of appropriate quantity will be excised from each wound at different time intervals and then at a gap of 7 days up to 21st post wounding day to estimate some biochemical parameters like hydroxyproline, glucosamine and protein.

- **Estimation of Hydroxyproline and Glucosamine**

  Some amount from each stored tissue sample will be subjected to acid hydrolysis by adding HCl to it in a tube which will be tightly sealed and autoclaved. The hydrolysate so produced will be used for estimating hydroxyproline and glucosamine.

- **Analysis of Proinflammatory Cytokines**

  Total protein of the skin homogenates will be determined by using Protein Assay. The concentrations of proinflammatory cytokines levels will be measured by using a commercially available multiplex cytokine kit according to the manufacturer's instructions (Samy *et.al.,* 2014).

  Markers for oxidative stress

- **Superoxide Dismutase Activity Assay**

  Total tissue superoxide dismutase (SOD) activity will be measured by using a commercially available kit that measures all three types of SOD (Dhall *et.al.,* 2014).

- **Alamar Blue assay**

  Cell proliferation assay kit will be used to measure cell proliferation rate and reduction in cell viability during apoptosis or necrosis. Cells will be plated out, in triplicate, into wells of a microtiter plate. Three control wells with medium alone will be used to provide blanks for absorbance readings. Treatment with various concentrations of silver nanoparticles will be added, followed by Alamar Blue to each well, including controls. When blue precipitate will clearly visible under the microscope, detergent reagent will be added to all wells, including control. The plate will be covered and left in the dark for overnight in 37°C incubator. The plate cover will be removed and the absorbance in each well will be measured in a microtiter plate reader. The average values from triplicate readings will be calculated and the blank will be subtracted from the average value.

Two-stage model for skin tumorogenesis

The tumor induction will be accomplished in two stages: Initiator and Promoter. The initiation will be done with the topical application of DMBA once. The promoter step will be done with the topical application of TPA, 3 times in a week.

Objective 4: To examine the anticancer activity of green synthesized metal nanoparticles

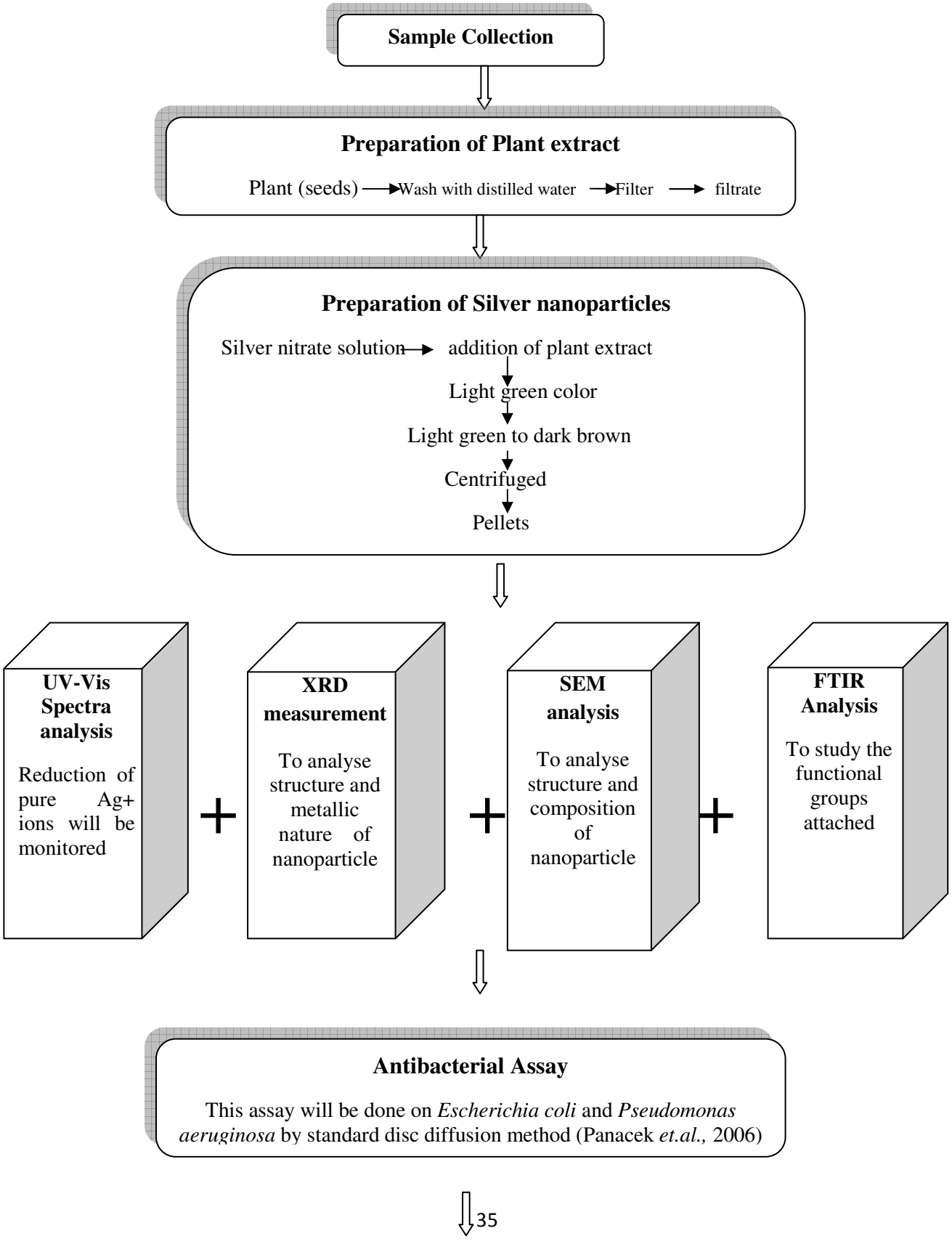
Study of anticancer activity against skin cancer:

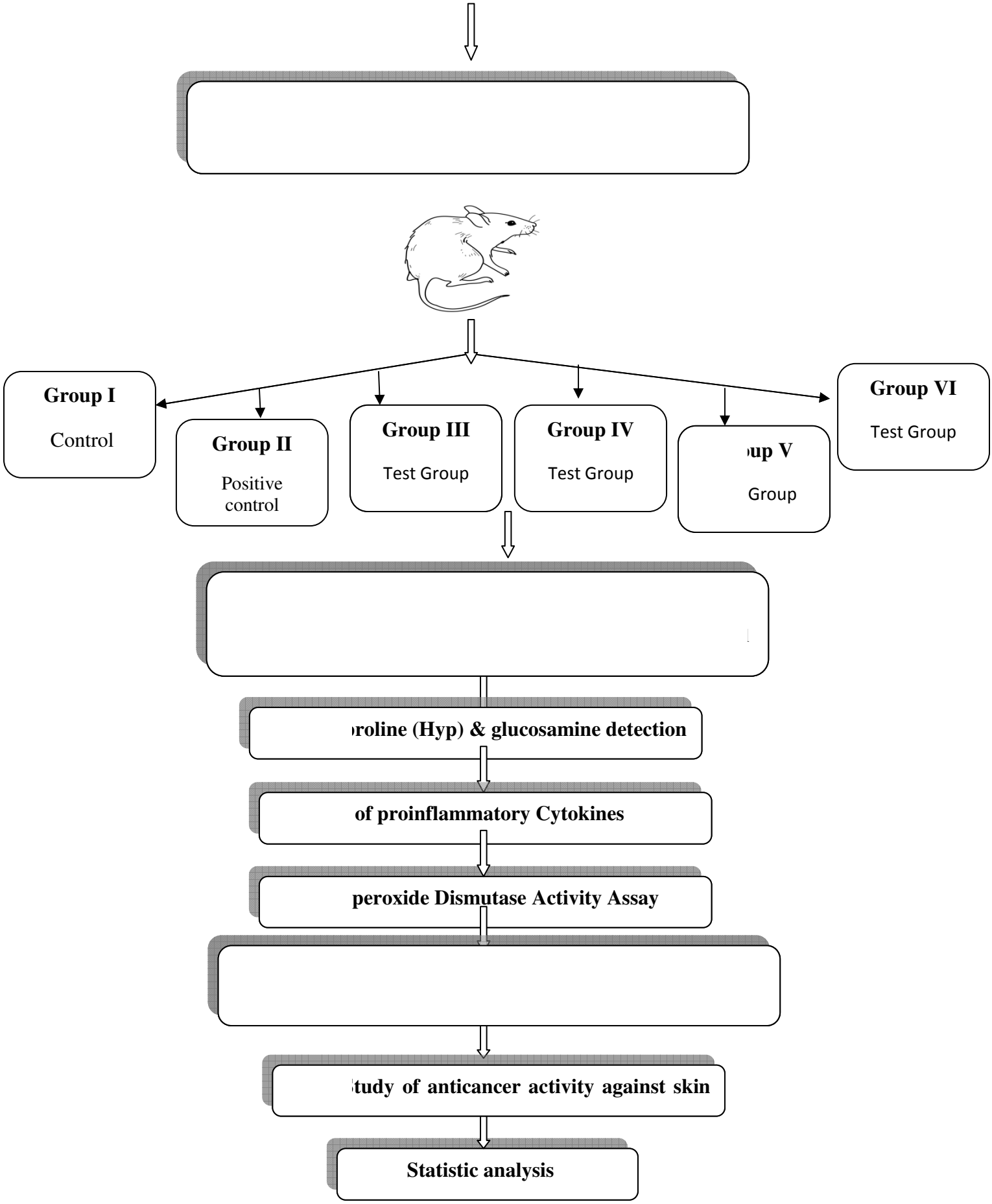
To study the anticancer activity of the biosynthesized AgNps, P13/AKT signaling pathway will be studied. The downregulation of AKT will serve as an indication of skin cancer protection. The treatment will be continued for 14 weeks. Along with it, biogenic AgNp Carbopol gel will be applied throughout for 14 weeks to monitor wound healing as well as anticancer activity (Mundi *et.al*., 2006).

Statistic analysis

The data will be statistically analyzed by using one way Analysis of Variance (ANOVA).

Plan of work


Sample Collection
Preparation of Plant extract
Plant (seeds)
Wash with distilled water
Filter
filtrate
Preparation of Silver nanoparticles
Silver nitrate solution
addition of plant extract
Light green color
Light green to dark brown
Centrifuged
Pellets
UV-Vis Spectra analysis
Reduction of pure Ag+ ions will be monitored
XRD measurement
To analyse structure and metallic nature of nanoparticle
SEM analysis
To analyse structure and composition of nanoparticle
FTIR Analysis
To study the functional groups attached
Antibacterial Assay
This assay will be done on Escherichia coli and Pseudomonas aeruginosa by standard disc diffusion method (Panacek et.al., 2006)


Group I
Control
Group II
Positive control
Group III
Test Group
Group IV
Test Group
up V
Group
Group VI
Test Group
roline (Hyp) & glucosamine detection
of proinflammatory Cytokines
peroxide Dismutase Activity Assay
tudy of anticancer activity against skin
Statistic analysis

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